Antibiotic susceptibilities, serotypes and auxotypes of *Neisseria gonorrhoeae* isolated in New Zealand

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Abstract

***Objective*—The aim of the study was to determine the distribution of auxotypes and serotypes and the prevalence of antibiotic resistance among New Zealand isolates of *Neisseria gonorrhoeae*.**

***Materials and methods*—A total of 486 gonococci isolated in 1988 were auxotyped, serotyped, and tested for susceptibilities to ten antibiotics.**

***Results*—The gonococci were susceptible to all the antibiotics tested except penicillin and tetracycline. Eleven (2·2%) produced beta-lactamase, one (0·2%) showed chromosomal penicillin resistance, and 18 (3·7%) were resistant to a low-level of tetracycline. Most of the gonococci belonged to six auxotypes. The three predominant auxotypes were arginine-requiring (Arg^-), non-requiring (NR), and arginine, hypoxanthine, uracil-requiring (AHU^-). The majority of the isolates belonged to serogroup IB and to six serovars. The most prevalent serovars were IB-3 and IB-1. There was an association between penicillin susceptibility and auxotype or serovar among non-penicillinase producing *N. gonorrhoeae* (PPNG) isolates.**

***Conclusions*—Antibiotic resistance, including penicillin resistance, remains uncommon among gonococci in New Zealand. Baselines have been established for future epidemiological studies using both auxotyping and serotyping.**

Introduction

The worldwide distribution of *Neisseria gonorrhoeae* and the emergence of new plasmid-mediated[1] and chromosomal resistances[2] emphasise the importance of epidemiological study of *N. gonorrhoeae* and surveillance of its susceptibility to antibiotics commonly used for therapy. Serotyping[3] and auxotyping[4] are powerful tools that can be used in the epidemiological study of gonococcal infections. To achieve the full potential from auxotype/serovar analysis, it is necessary to have data on the auxotypes and serovars of strains within geographical areas. Previous national surveys in 1976[5] and 1980 (Green, unpublished observations) showed low rates of antibiotic resistance among gonococci in New Zealand. In this study, we report on the distribution of auxotypes and serotypes, and the prevalence of antibiotic resistance among New Zealand isolates of *N. gonorrhoeae* in 1988.

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Accepted for publication 6 April 1992

Materials and methods

Bacterial strains

A total of 486 *N. gonorrhoeae* from genital sites were tested. The isolates were referred to the New Zealand Communicable Disease Centre by 39 laboratories throughout New Zealand in 1988. Isolates were identified as *N. gonorrhoeae* as described previously.[6] All isolates were cultured on GC Medium (Difco Laboratories), supplemented with 1% haemoglobin (Difco) and 1% Isovitalex (BBL). Organisms were stored at −70°C in Tryptic Soy Broth (Difco) containing 15% (v/v) glycerol.

Antibiotic susceptibility tests

The antimicrobial susceptibilities were determined by an agar dilution method[7] using Isosensitest agar with 8% lysed horse blood and an inoculum of 10^4 cfu. The plates were read after 18–20 h incubation at 35°C with 5% CO_2. The antimicrobials tested were Augmentin, cefotaxime, cefoxitin, ceftriaxone, cefuroxime, ciprofloxacin, co-trimoxazole, penicillin, spectinomycin, and tetracycline. Beta-lactamase production was tested by a chromogenic cephalosporin method.[8]

Plasmid profiles

Plasmid profiles were determined for the penicillinase-producing *N. gonorrhoeae* (PPNG) isolates. Plasmid DNA was extracted by a rapid method,[9] electrophoresed on 0·7% agarose and visualised by ethidium bromide staining.

Serotyping

The isolates were serotyped by a coagglutination method using a panel of six Protein IA-specific and six Protein IB-specific monoclonal antibodies.[3] Serovars were designated by the nomenclature of Knapp *et al.*[3] In accordance with others,[10 11] serovars which belong to IA-1 and IA-2 or to IB-5 and IB-7 were considered as single serovars.

Auxotyping

All isolates were tested for their nutritional requirement for arginine, hypoxanthine, uracil, proline, and methionine by modification of a previously described method[12] using Gonococcal Genetic Medium (GGM)[13] as the defined minimal medium.

Statistical analysis

Differences in penicillin susceptibility of the predominant serovars and auxotypes of non-PPNG isolates were determined using the chi square test.

Table 1 Susceptibility of 486 gonococci to ten antibiotics

	MIC (mg/l)					
	Non-PPNG (n = 475)			PPNG (n = 11)		
Antibiotic	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Augmentin*	0·004–1	0·12	0·25	0·5–2	1	2
Cefotaxime	0·004–0·06	0·004	0·008	0·004–0·008	0·004	0·008
Cefoxitin	0·06–2	0·12	0·25	0·25–1	0·5	1
Ceftriaxone	0·004–0·06	0·004	0·004	0·004	0·004	0·004
Cefuroxime	0·004–1	0·016	0·03	0·016–0·06	0·06	0·06
Ciprofloxacin	0·004–0·008	0·004	0·004	0·004–0·008	0·004	0·008
Co-trimoxazole†	0·06–1·0	0·12	0·5	0·12–1·0	0·5	1·0
Penicillin	0·004–1·0	0·03	0·12	2·0–64	16	32
Spectinomycin	2–32	8	8	4–32	8	8
Tetracycline	0·06–2	0·25	1	1–2	2	2

* Expressed as concentration of amoxycillin
† Expressed as concentration of trimethoprim in trimethoprim:sulphamethoxazole of 1:19
MIC_{50}: MICs required to inhibit 50% of isolates
MIC_{90}: MICs required to inhibit 90% of isolates

Results

Antibiotic susceptibility

The gonococcal isolates were susceptible to all the antibiotics tested except penicillin and tetracycline. Eleven (2·2%) produced beta-lactamase, one (0·2%) showed chromosomal penicillin resistance, and 18 (3·7%) were resistant to a low-level of tetracycline. The antibiotic susceptibilities of the 475 non-PPNG and 11 PPNG isolates are shown in table 1.

Seven (64%) of the PPNG isolates were tetracycline-resistant (MIC > = 2 mg/l). No plasmid mediated high-level tetracycline resistance (MIC ≥ 16 mg/l) was seen. Plasmid analyses showed that eight isolates carried the Asian type beta-lactamase plasmid (4·5 Md), two isolates the Rio/Toronto type plasmid (3·0 Md), and one isolate the African type plasmid (3·2 Md). Transfer plasmids (24·5 Md) were carried by all the PPNG isolates except two with the Asian type plasmid.

Among the non-PPNG isolates, 11 were resistant to a low-level of tetracycline and one showed chromosomal penicillin resistance (MIC ≥ 1 mg/l). The distribution of the penicillin MICs of the non-PPNG isolates (fig) was bimodal. Of the non-PPNG isolates, 50·9% were penicillin-sensitive (MIC ≤ 0·03 mg/l) and 48·9% were penicillin less sensitive (MIC 0·06–0·5 mg/l).

Serotyping

Serotyping of the 11 PPNG isolates showed that one belonged to serogroup IA and ten to serogroup IB. Six serovars were found among the PPNG isolates; four isolates were IB-1 and three isolates were IB-3.

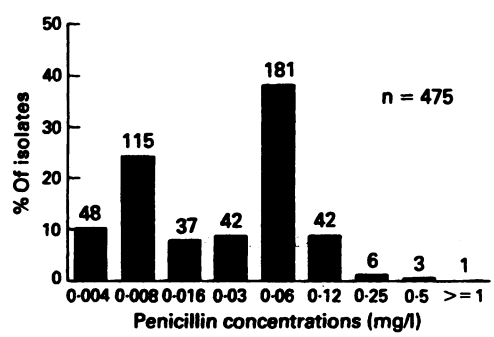

Fig Distribution of penicillin MICs of 475 non-PPNG isolates.

Sixteen serovars, four IA and 12 IB specific serovars, were represented among the 475 non-PPNG isolates serotyped. However, most (91·8%) of the isolates belonged to six predominant serovars (table 2). A total of 447 (94·1%) isolates were IB serovars. The most prevalent serovars were IB-3 and IB-1 which accounted for 71·2% of the isolates.

The distribution of the six predominant serovars among the non-PPNG isolates that were either penicillin-sensitive (MIC ≤ 0·03 mg/l) or penicillin less sensitive (MIC 0·06–0·5 mg/l) is shown in table 2. There was a significant ($p < 0·001$) association between serovar and penicillin susceptibility. There were more serovar IB-14, IB-5/7 and IB-3 isolates among the penicillin less sensitive group and more serovar IB-1 isolates among the penicillin-sensitive group.

Auxotyping

Six auxotypes were represented among the 11 PPNG isolates. Four PPNG isolates were Pro^- (proline-requiring) and two were NR (non-requiring). Of the 475 non-PPNG isolates, 454 (95·6%) belonged to six predominant auxotypes (table 3). A total of 14 auxotypes were found. The most prevalent auxotypes were Arg^- (arginine-requiring), AHU^- (arginine, hypoxanthine, uracil-requiring) and NR. There were 23 isolates that required arginine, hypoxanthine, uracil and methionine. Five of these isolates were inconsistent in their requirement for methionine.

Table 3 shows the distribution of the six predominant auxotypes among non-PPNG isolates that were either penicillin-sensitive (MIC ≤ 0·03 mg/l) or penicillin less sensitive (MIC 0·06–0·5 mg/l). There was a significant correlation ($p < 0·001$) between penicillin susceptibility and auxotype. There were more NR and Arg^- isolates among the penicillin less sensitive group; and more AHU^-, $AHUM^-$, and $ProArg^-$ isolates among the penicillin-sensitive group.

Auxotype/Serovar classes

There were ten auxotype/serovar (A/S) classes among the 11 PPNG isolates and 60 A/S

Table 2 Predominant serovars among 475 non-PPNG isolates

Serovar	Number (%) of total isolates	Number (%) of total isolates that were: Penicillin-* sensitive	Penicillin less† sensitive
IB-3	216 (45·5)	94 (19·8)	121 (25·5)
IB-1	122 (25·7)	93 (19·6)	29 (6·1)
IB-14	32 (6·7)	5 (1·0)	27 (5·7)
IB-2	26 (5·5)	17 (3·6)	9 (1·9)
IA-1/2	22 (4·6)	16 (3·4)	6 (1·2)
IB-5/7	18 (3·8)	2 (0·4)	16 (3·4)
Totals	436 (91·8)	227 (47·8)	208 (43·8)

* Penicillin MIC ≤ 0·03 mg/l
† Penicillin MIC = 0·06–0·5 mg/l

Table 3 Predominant auxotypes among 475 non-PPNG isolates

Auxotype	Number (%) of total isolates	Number (%) of total isolates that were: Penicillin-* sensitive	Penicillin less† sensitive
Arg	178 (37·5)	42 (8·9)	136 (28·6)
AHU	100 (21·1)	97 (20·5)	3 (0·6)
NR	87 (18·3)	29 (6·1)	58 (12·2)
ProArg	39 (8·2)	28 (5·9)	10 (2·1)
Pro	27 (5·7)	10 (2·1)	17 (3·6)
AHUM	23 (4·8)	23 (4·8)	0 (0)
Totals	454 (95·6)	229 (48·3)	224 (47·1)

* Penicillin MIC ≤ 0·03 mg/l
† Penicillin MIC = 0·06–0·5 mg/l
Arg = arginine-requiring; AHU = arginine, hypoxanthine, uracil-requiring; NR = non-requiring; ProArg = proline, arginine-requiring; Pro = proline-requiring; AHUM = arginine, hypoxanthine, uracil, methionine-requiring.

classes among the 475 non-PPNG isolates. The distribution of the non-PPNG isolates among the six predominant auxotypes and serovars is shown in table 4. The main A/S class among the non-PPNG isolates was Arg$^-$/IB-3 which accounted for 99 (20·3%) of the isolates tested.

The single isolate with chromosomal penicillin resistance was in the A/S class ProArg$^-$/IB-3. Eighty-seven per cent of the Arg$^-$/IB-14, 85% of NR/IB-5/7, and 84% of Arg$^-$/IB-1 isolates were penicillin less sensitive.

Discussion

Comparison of the antibiotic susceptibilities of gonococci in the present study with the earlier national surveys in 1976[5] and 1980 (Green, unpublished observations) shows that there have been no appreciable changes in antibiotic susceptibilities except for penicillin. In the 1980 study, 4·7% of 318 isolates had penicillin MICs of 1–4 mg/l compared with 0·2% in the present study. However, it is difficult to assess the significance of the apparent decrease as a different sensitivity testing medium was used in the earlier studies. In the 1976 and 1980 studies, the medium used was Proteose No 3 agar supplemented with 1% haemoglobin and 1% Isovitalex. In a comparative study[14] of the effect of media on penicillin MICs, it was shown that Isosensitest agar with 8% lysed horse blood tended to give lower readings than other media tested.

Our results show that gonococci isolated in New Zealand are very susceptible to most antibiotics; just penicillin resistance and low-level tetracycline resistance were identified and only among a small percentage of the isolates surveyed. There were no PPNG isolates in the 1976 survey although the first PPNG isolated in New Zealand was confirmed shortly after the survey ended. There were 12 (3·2%) PPNG isolates in the 1980 survey and 11 (2·2%) PPNG isolates in the present study. This contrasts with reported PPNG isolation rates of 40–50% in parts of Asia[15,16] and 40-80% in parts of Africa.[17,18]

Similarly, our rate of chromosomal penicillin resistance at 0·2% is considerably lower than Britain,[19] the United States,[20] and Australia.[21] It has been reported that in the Far East there is a high prevalence of chromosomal penicillin resistance. In one study, 53% of non-PPNG isolates isolated in Bangkok have penicillin MICs ≥ 1 mg/l.[16]

It was notable that Arg$^-$, AHU$^-$ and NR strains predominated in New Zealand. Studies have shown that the predominant auxotypes in the Netherlands,[22] Britain,[19] Greece,[23] Jamaica,[24] Korea,[25] Chile,[26] and Argentina[27] are NR and Pro$^-$. We showed that 94·1% of our isolates belonged to serogroup IB. Similarly, several studies[3,9,24,25] have shown the predominance of serogroup IB strains in many geographical areas.

Our results showed an association between auxotype or serotype and penicillin sensitivity. The correlation of AHU$^-$ strains with penicillin susceptibility has been previously shown.[28,29] Our study confirmed the significant association of the AHU$^-$ auxotype with penicillin sensitivity. We further showed the significant correlation of the AHUM$^-$ and ProArg$^-$ auxotypes with penicillin sensitivity. Association between serovar IB-5/7 and chromosomal penicillin resistance has been previously shown.[9] Our results show that serovars IB-14, IB-5/7 and IB-3 predominated among the penicillin less sensitive strains.

It is of note that our results showed that 88% of the AHU-isolates belonged to serogroup IB.

Table 4 Distribution of 475 non-PPNGs among six predominant serovars and six predominant auxotypes

Serovar	Auxotype: Arg	AHU	NR	ProArg	Pro	AHUM	Others	Total
IB–3	99	36	58	3	12	4	4	216
IB–1	25	48	5	16	3	16	9	122
IB–14	31	1						32
IB–2	8	1	1	11	2		3	26
IA–1/2	2	12		5		3		22
IB–5/7	3		13				2	18
Others	10	2	10	4	10		3	39
Total	178	100	87	39	27	23	21	475

Arg = arginine-requiring; AHU = arginine, hypoxanthine, uracil-requiring; NR = non-requiring; Pro = proline-requiring; AHUM = arginine, hypoxanthine, uracil, methionine-requiring.

With the exception of another study[11] where 46% of the AHU-isolates were AHU/IB strains, other studies[3 30] have shown that most AHU-isolates belonged to serogroup IA.

There were too few PPNG isolates to show adequately any correlation between serovars and auxotypes. However, the wide variety of auxotypes, serovars and plasmid types among 11 isolates of PPNG suggest the importation of strains from other countries rather than local circulation of a few strains.

In conclusion, we showed that antibiotic resistance, including penicillin resistance, remains uncommon among gonococci in New Zealand. We have also established baselines for future epidemiological studies using auxotype/serovar typing.

This paper is published with the authority of the Director-General of Health, New Zealand.

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